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FACSIMILE COVER LETTER

To:	*Examiner*: Shin Lin Chen *Group*: 1632
Firm:	U.S.P.T.O.
Facsimile:	571-273-0726
From:	Marilyn Matthes Brogan
Date:	February 24, 2005
Re:	Applicant: Loerz et al **Application No.:** 09/674,824 **Filed:** November 6, 2000 **For:** NUCLEIC ACID MOLECULES WHICH CODE FOR ENZYMES DERIVED FROM WHEAT AND WHICH ARE INVOLVED IN THE SYNTHESIS OF STARCH Our Ref. No. 514413-3848
Number of Pages: (including cover page)	2

If you do not receive all pages or are unable to read the transmission, please call and ask for Anna (Ext. 2068).

Dear Mr. Chen:

Further to our earlier conversation of today, attached is a copy of page 30 of the specifications to the above referenced matter.

Best regards.

Very truly yours,

Marilyn Matthes Brogan

PAGE 1/2 * RCVD AT 2/24/2005 4:00:42 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/24 * DNIS:2730726 * CSID:212 588 0500 * DURATION (mm-ss):00-58

The method for transforming immature wheat embryos was developed and optimized by Becker and Lörz (D. Becker and H. Lörz, Plant Tissue Culture Manual (1996), B12: 1 to 20).

In the experiments described hereinbelow, the procedure developed by Becker and Lörz (loc. cit.) was adhered to.

For the transformation, ears with caryopses of developmental stage 12 to 14 days after anthesis were harvested and surface-sterilized. The isolated scutella were plated onto induction medium #30 with the embryo axis orientated towards the medium.

After preculture for 2 to 4 days (26°C, in darkness), the explants are transferred to medium #39 for the osmotic preculture (2 to 4 h, 26°C, in the dark).

For the biolistic transformation, approx. 29 µg of gold particles onto which a few µg of the target DNA had previously been precipitated were employed per shot. Since the experiments carried out are cotransformations, the target DNA added to the precipitation batch is composed of the target gene and a resistance marker gene (bar gene) in the ratio 1:1.

4. DIG labeling of DNA fragments

DNA fragments employed as screening probes were labeled via a specific PCR with the incorporation of DIG-labeled dUTP (Boehringer Mannheim, Germany).

Media solutions used in the examples:

20 × SSC	175. 3 g NaCl
	88.2 g sodium citrate
	twice-distilled H_2O to 1000 ml
	10 N NaOH to pH 7.0

Plasmid pTaSSI 8/1 was deposited at the DSMZ in Braunschweig, Federal Republic of Germany, as specified in the Budapest Treaty under the No. DSM 12794.

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